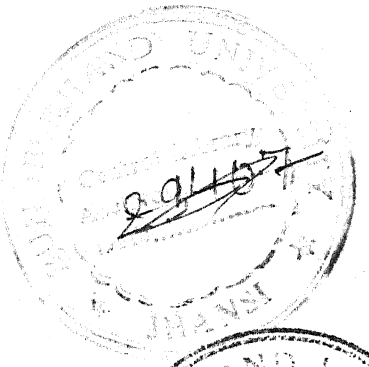
A CLINICO-MICROBIOLOGICAL STUDY OF ACUTE DIARRHOEA IN CHILDREN

THESIS
OF
DOCTOR OF MEDICINE
(PAEDIATRICS)



BUNDELKHAND UNIVERSITY
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AJAY KUMAR TIWARI

C E R T I F I C A T E

This is to certify that the work entitled "A CLINICO-MICROBIOLOGICAL STUDY OF ACUTE DIARRHOEA IN CHILDREN", which is being submitted as a thesis for M.D.(Paediatrics) examination, 1992 of Bundelkhand University, has been carried out by Dr. Ajay Kumar Tiwari in the department of Pediatrics and Microbiology, M.L.B. Medical College, Jhansi.

He has put in the necessary stay in the department as per university regulations.

Dated: 25.10.91

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C E R T I F I C A T E

This is to certify that the work entitled "A CLINICO-MICROBIOLOGICAL STUDY OF ACUTE DIARRHOEA IN CHILDREN" which is being submitted as a thesis for M.D.(Pediatrics) examination, 1992 of Bundelkhand University by Dr. AJAY KUMAR TIWARI, has been carried out under my guidance and supervision. The techniques and statistics mentioned in the thesis were actually undertaken by the candidate himself. The observations recorded were checked and verified by me from time to time.

Dated:

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(GUIDE)

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The results have been checked by me from time to time.

Dated:

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A C K N O W L E D G E M E N T S

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Dated:

Ajay Tiwari

(Ajay Kumar Tiwari)

C O N T E N T S

I N T R O D U C T I O N

INTRODUCTION

From the worldwide perspective acute diarrhoeal diseases in children constitute a cardinal health problem in developing countries, where it is associated with high morbidity and mortality. Studies from developing countries have shown that children under 5 years of age experience on an average 2-3 episodes of diarrhoea every year(Synder and Marson, 1982). The consequence of diarrhoeal episodes in terms of childhood nutrition, overall health and survival are substantial.

Children in underprivileged communities often live in grossly contaminated environment with substandard water supply, unsafe sewage disposal, low standard of community and personal hygeine. All these factors exposes children to high risk of acquiring acute diarrhoeal disease. An extra problem arises when breast feeding is substituted by bottle feeding which again poses an extra risk of acquiring gastro-intestinal infection. Association with poverty and underdevelopment with diarrhoeal disease has led some to the view that amelioration will only follow successful attempts to raise the general living standard of communities in which diarrhoea is prevalent.

Most of aucte diarrhoeas are infectious in origin. Till the recognition of role of viruses and certain bacteria in causation of acute diarrhoea no pathogens were identified in majority of patients. With the introduction of

laboratory method to identify and isolate different viruses and bacteria, now it is possible to identify causative agents in over two thirds of cases of diarrhoea. A wide variety of infectious agents is bacteria, viruses and parasites are implicated in aetiology of acute diarrhoea.

Among the many etiologic agents that have been associated with pediatric diarrhoeal diseases in developing countries relatively few account for most diseases of public health importance (Levine, 1986). These are rotaviruses enterotoxigenic E. coli, Enteropathogenic E. coli, Shigella and Vibrio cholerae 01. A number of virulence factors in enteropathogens enables them to produce intestinal infection and diarrhoeal disease. Crypt cell proliferation, cellular invasion, elaboration of enterotoxins and cytotoxins and enteroadhesion are mechanisms by which enteropathogens cause diarrhoeal disease. Young children are related, both to increased exposure and age specified alterations in susceptibility and response to enteropathogenes.

Rotavirus is a major cause of severe dehydrating diarrhoea in young children in both developing and developed countries (WHO, 1989). Rotavirus diarrhoea is most common in children 6-24 months of age. It accounts of 20-40% of severe diarrhoea. In developing countries bacterial enteropathogens appear to be important cause of diarrhoea.

Aetiological studies of diarrhoeal disease are necessary to define the relative importance of various

enteropathogens in a population and to direct therapeutic and preventive efforts for reduction of impact of these illnesses in a population. However, for care of individual patient precise aetiological diagnosis is not essential. As oral rehydration therapy is the treatment of diarrhoeal disease of all ages and causes (WHO, 1983). Enteric infection causing diarrhoea are generally self limiting. However, diarrhoea caused by Shigella, V. cholera, Giardia and E. histolytica are effectively treated by antimicrobial drugs and only patientswith these infections would clearly benefit from aetiological diagnosis and specific therapy.

The prevalence of different enteropathogens vary with geographical areas and epidemiological setting of the study. Since no study regarding relative contribution of various enteropathogens in aetiology of acute diarrhoea has been carried out in Bundelkhand region, it was thought worthwhile to conduct the present study and it was aimed to find out :-

1. Aetiology of acute diarrhoea in children under 5 years of age attending Pediatrics out patient Clinic of M.L.B. Medical College, Jhansi.
2. To study the clinical profile of acute diarrhoea.
3. To study the difference, if any, in aetiology of diarrhoea in breast fed and non-breast fed and undernourished children.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

In 1980, in the developing countries an estimated 5 million children under 5 years of age died as a consequence of diarrhoeal disease. The deaths were an outcome of some 1000 million episodes that occurred among 338 million children in this age group and undoubtedly more frequently in poorer families (WHO, 1983). 3.5 million children still die due to diarrhoeal disease in developing countries (WHO, 1989). The diarrhoeal morbidity rate has been found to be higher in first two years of life. In less than 5 years' age group attack rate of diarrhoeal diseases ranges from 2-3 per child per year in many developing countries (Gordon et al, 1963; Synder and Merson,1982; Reddiah and Kapoor, 1991). Mata et al in his study in rural Guatemala showed a higher incidence,7.9 per child per year probably because of much closer follow up.

Most diarrhoeas occurring in general population are of an infectious nature. The recognition of role of certain viruses and bacteria as causes of diarrhoea now makes it possible to identify the causative agent in over 2/3rds of the diarrhoea presenting at treatment centre (WHO, 1980). This dramatic development reverses the situation present 2 decades ago when the aetiology in over 80% of these cases remained unknown.

A wide variety of infectious agents including viruses, bacteria and parasites cause diarrhoea in

susceptible host. Rotavirus, Norwalk agent, Adeno viruses, calci virus, corona virus and astro virus are notable among viruses. Bacteria like Escherichia Coli - Enterotoxigenic E. Coli (E.T.E.C.), Enteropathogenic E. Coli (EPEC), enteroinvasive E.Coli (EIEC), Enteroadherent E.Coli(EAEC) and enterohaemorrhagic E.Coli Shigella - nontyphoid salmonella, Camoylobacter jejuni, Vibrio cholerae. 01 and non 01, Vibrio parahaemolyticus, Aeromonas hydrophila, Plesiomonas shigelloides, Pseudomonas aeruginosa, Klebsiella pneumoniae, Yersinia enterocolitica, Clostridium difficile and C. perfringens, Arizona and Edward Siella have been incriminated in the aetiology of diarrhoea.

Among parasites, Entamoeba histolytica, Giardia lamblia and cryptosporidium have been found to be associated diarrhoea.

The commonest bacteria responsible for diarrhoea in children are EPEC, ETEC and Shigellae (Sanyal et al, 1977; Mahalanbis, 1975; Black et al, 1981). Vibrio cholerae becomes important in endemic areas.

Rotavirus is the most important cause of viral diarrhoea. It is responsible for upto 50% of acute diarrhoea in childre in 6-24 months group visiting treatment facilities and 5-10% of all diarrhoeas in the community (WHO, 1980).

Amongst parasites E. histolytica and G. lamblia are dominant agents (Sanyal, 1977 and Mahalanbis, 1977).

Bacterial enteropathogens express a wide range of virulence factors or specific mechanism that enable

them to overcome host defence mechanism. They may produce diarrhoea by one or more of the following mechanisms.

1. Invasion of mucous with inflammation and ulceration.
2. Elaboration of cytotoxins that greatly alter the mucosal surface.
3. Elaboration of protein enterotoxins that greatly alter the intestinal salt and water balance without affecting mucosal morphological features.
4. Colonization and adherence to the surface.

BACTERIAL PATHOGENS GROUPED BY PATHOGENIC MECHANISM

INVASIVE	
Shigella	Salmonella
Y. enterocolica	Campylobacter jejuni
V. parahaemolyticus	
CYTOTOXIC	
Shigella	E.P.E.C.
Enterohaemorrhagic E.Coli	C. difficile
TOXIGENIC	
E.T.E.C.	Vibrio cholerae 01 and Non 01
K. pneumoniae	Aeromonas hydrophila
	Plesiomonas shigelloides
ADHERENT	
E.P.E.C.	Enterohaemorrhagic E.Coli

VIRAL DIARRHOEA

For many years viruses were suspected by paediatricians of causing a significant proportion of episodes

of childhood gastroenteritis which previously would have been undiagnosed. This suspicion seems to have been well founded, because over recent years, viral infections have become accepted as a major cause of diarrhoeal disease, particularly in children below 2 years of age (Cukor and Blacklow, 1984).

Rotavirus is best known of these viral agents, but there are many others including Norwalk agents adenoviruses, coronaviruses, calciviruses, small round viruses(SRV).

ROTAVIRUS

Rotavirus is probably the commonest virus which infects humans and is responsible for a great deal of vomiting and diarrhoeal illness in infants and young children. Rotavirus is the major cause of severe dehydrating diarrhoea in both developed and developing countries. The organism is responsible for 40-60% diarrhoea cases requiring hospitalization in developed countries and it accounts for 20-40% of severe diarrhoeas among children in developing world (WHO, 1989).

Human rotavirus was first visualized in 1973 in Australia in duodenal biopsy specimen of infants and young children suffering from non bacterial gastroenteritis (Bhishop et al, 1973) by electron microscopic examination. Later they were easily recognised in stool specimen directly by E. M. (Flewett, 1973). In relatively short time thereafter laboratories all over the world reported the presence of rotavirus in the stool specimen from infants

and young children with acute diarrhoea.

Rotaviruses are members of the family Reoviridae, the prefix "rota" means wheel which the virus resembles morphologically. The virus has an average diameter of 70 nm with an inner core of RNA, surrounded by a double shield capsid. It contains a genome of 11 segments of double stranded RNA, which is capable of reassortments.

Rotavirus disease is predominantly a disease of under 2 years old with peak incidence about 7-12 month of age (Brandt et al, 1983; Rodreugz et al, 1977; WHO Sci. group, 1980). The average incubation period is about 1 to 3 days (Davidson et al, 1975). The onset of illness is characterized by severe watery diarrhoea vomiting and low grade fever. Vomiting is particularly common feature of rotavirus diarrhoea in children as compared with occurrence of this symptom in gastroenteritis due to other causes (Rodriquez et al, 1977). The mean duration of rotaviral illness in 5 to 8 days. The concentration of rotavirus in the faeces reaches its maximum peak shortly after the onset of illness and diminishes gradually until 9 to 10 days.

The stool are usually liquid and without blood Faecal leucocytes are uncommon and mucous is some times seen (Rodriquez et al, 1977; Heiher et al, 1978). Common clinical feature of rotaviral illness is isotonic dehydration, occurring in 40-83% of the cases. The level of dehydration is usually less than 5% but can be greater

in severe cases.

In temperate climate most episodes occur during winter season. In tropics rotavirus enteritis tends to occur throughout the year although seasonal variation have been reported.

In a usual case of rotavirus enteritis vomiting is a prominent early feature and in many cases precedes the onset of watery diarrhoea. Mucous is found in stools in upto 25% cases, blood is rate. Mild temperature elevation is present in 30-50% cases. Upper respiratory symptoms have been reported (WHO, 1980). Symptoms of upper respiratory tract infection have been reported to be associated with rotavirus diarrhoea (Rodriquez et al, 1977; Lewis et al, 1979). But rotavirus has never been demonstrated to infect respiratory tract (Lewis et al, 1979). The association may be coincidental.

The importance of rotavirus illness has been well documented in developed countries. In the developed countries rotavirus is responsible for about half of the hospitalized cases of acute diarrhoeal illness in this age group (Brandt et al, 1983; Kapikian et al, 1976 and Rodriquez et al, 1977).

In studies from Bangladesh by Black et al(1981) in moderately severe to severe gastroenteritis, rotaviruses were found to be most important pathogen below 2 years of age and younger, 46% of such patients shed

agent. Bacterial agents were detected more frequently than rotavirus among children of 2 years of age and older.

The greater degree of dehydration was observed in a Bangladesh study, during rotavirus diarrhoea 44% of the children experienced dehydration and 30% visited the treatment centre. In contrast ETEC diarrhoea only 14% cases below 2 years of age had dehydration and 5% visited treatment centre both ($p < 0.001$) (Black et al, 1981).

Although rotavirus infection has been widely accepted as a major cause of gastroenteritis in infants and young children. Asymptomatic excretion is not uncommon. Champasur et al (1984) found asymptomatic virus shedding in 71% neonates, 50% of 1 to 6 months old infants, and 26% of children aged 7 to 24 months in a prospective study of patients admitted in Paris.

Infection with rota virus induces seroconversion and elevation of antibody titre. The prevalence of rota virus antibody is high in newborns due to transfer of passive antibody from the mother then it diminishes in first 6 months of life and again high titre are present by 2 to 3 years of age and maintained throughout the life. In a study in Washington, rotavirus antibody was detected in over 90% of children by third year of age (WHO, 1980).

STUDIES IN INDIA

In India first study was conducted in Vellore by Holmes et al (1974) first of its kind from India

detected rotavirus in 3 of the five children between 4-22 months age group.

In another study from Vellore Maiya et al (1977) reported 26% hospitalised diarrhoea cases to be rotavirus positive. This study also showed seasonal pattern of diarrhoea associated with rotavirus.

Paniker et al (1977) reported an epidemic diarrhoea in north Kerala caused by rotavirus. They demonstrated rotavirus particles by E.M. examination in all the 10 faecal samples of patients 7 months - 4½ years of age old. All of them were admitted with diarrhoea and vomiting.

In 1981 another epidemic of rotavirus diarrhoea was reported from Manipur by Sen Gupta et al (1981).

Yet another hospital based study from Calicut, by Panikar et al (1982) carried out over a period of 16 months showed high prevalence of rotavirus diarrhoea in infants and young children. In this study rotavirus was detected by electron microscopy in stool of 70.7% of the children. Prevalence of virus was high nearly 100% of cases examined during Nov., to January and Lowest in May. Prevalence of rotavirus was high (75.1%) in infants from 6-23 months of age.

Samantray et al (1982) studied the prevalence and seasonal occurrence of rotavirus diarrhoea among 212 preschool children with diarrhoea from a community and 99 cases from hospital in Delhi. The detection rate of

rotavirus was 21.2% in the community and 32.2% in hospital cases. There were no conspicuous peaks in the detection rates through out the year, though a relatively lower peak was detected during July through September. The clinical features of rotavirus diarrhoeal disease did not show any significant difference.

Bhan et al (1987) in their study of etiology of acute diarrhoea in 204 children below 5 years of age detected rotavirus in 20.6% cases, from an out patient dispensary.

Bhat et al (1985) studied the etiological role of rotaviruses in acute diarrhoeal illness in 0-5 years children admitted to a pediatric ward. Rota virus accounted for 16.3% of acute diarrhoea, peak detection rate was found in Jan. and Feb.,

Mohandas et al (1987) detected rotavirus in 18% of children below 2 years who presented with acute diarrhoea in an out patient clinic in Vellore. Syndrome of watery diarrhoea and vomiting was highly associated with rotavirus infection. Upper respiratory tract infection and fever was not significantly associated with rotaviral diarrhoea in this study.

An epidemiological study of rotavirus infection initiated by the National Institute of Virology (NIV) Pune in January, 1981. For the base line data, hospital records in a large Government teaching hospital in Pune, were screened retrospectively for years 1979-80. Stool

specimens from a representative sample of 213 hospitalized children with diarrhoea and from an equal number of non diarrhoeal controls were investigated, both by EM examination and ELISA for detection of viruses. Rotavirus were observed in 28.6% of diarrhoeal patients less than 12 years of age and only 1.4% of non diarrhoeal controls. The prevalence of rotavirus was much higher 96.7% in children aged from 6-24 months. Seasonwise analysis showed significant number of children with rotavirus during coller months of the year (ICMR Bulletin, 1986).

There are 4 serotypes of rotaviruses designated 1 to 4. While they all cause disease, serotype-1 appears to be most common cause of epidemic rotavirus disease in countries with temperate climate. Information on distribution of rotavirus according to serotype in developing countries is limited (WHO, 1989).

It has been estimated that effective rotavirus vaccine could reduce all diarrhoeal deaths by 30% in the age group of 6-24 months and overt 500,000-1000,000 deaths in children annually (WHO, 1989).

E. COLI

E.coli, the most familiar and numerous organism in the faecal flora of the humans and other animals, is a well known opportunistic pathogen when outside its normal etiological etiological niche. Although E. coli has been suspected also for many years as a possible etiology of

diarrhoeal disease with its habitate, this association was not conclusively proved until mid 1940's when hospital nursery outbreaks of severe lethal diarrhoea occurred which were shown to be due to single serotype of E. coli (Bray, 1945; Taylor et al, 1949).

In the evaluation of these outbreaks, serotyping was shown to be an excellent epidemiological marker for recognition of bacterial virulence and these organisms were termed enteropathogenic E.coli (EPEC). Although the mechanisms whereby these organisms cause diarrhoea were not known. Enterotoxins were suspected as being virulence factor as early as 1956 by De et al and later by Taylor and Bettleehiem (1966) but their existence could not be conclusively demonstrated. In mid to late 1960's enterotoxin producing E. coli was first isolated from domestic young animals with severe diarrhoea. Shortly thereafter strains of ETEC were described in the etiology of severe cholera like illness in humans in Calcutta (Sack et al, 1971).

The understanding of pathogenesis of diarrhoea due to E. coli has undergone considerable change in past two decades. Today it has been accepted practice to identify and to distringuish the E. coli into 4 types (1) Enterotoxigenic E. coli (2) Enteropathogenic E.coli (3) Enteroinvasive E. coli (4) Enterohaemorrhagic E.coli.

ENTEROTOXIGENIC E. COLI

ETEC can produce one or both of the two recognised enterotoxins : a heat labile enterotoxin that is immunologically related to cholera toxin (LT) and a heat stable , low molecular weight, non antigenic enterotoxin (ST), thus there are LT strains, ST strains and LT/ST strains. The genetic material that controls the production of these enterotoxins is located on plasmids and thus relatively easily transferable to recipient strains (WHO Scientific group, 1980).

Like cholera toxin, LT induces a secretory diarrhoea through increased production of adenylase cyclase and with a subsequent increase in cyclic-AMP. In case of ST, it has been shown to cause secretory diarrhoea by stimulating guanyl cyclase activity, leading to persistent increase in cyclic GMP.

We now know that these organisms are a major cause of diarrhoeal illness in children in developing part of world (WHO Scientific group, 1980). Studies carried out in developed countries notably in USA, Canada, Japan and England have shown that ETEC are an infrequent cause of diarrhoeal illness(Merson and Black, 1980).

During past decade enterotoxogenic E.coli has appeared as one of the most prevalent diarrhoeagenic agent in children in developing world. However, isolation rates of ETEC show a wide variance in different

studies (Paul et al, 1980; Sarkar et al, 1980; Ganguly et al, 1980; Black et al, 1981, Panhotra et al, 1980; Stoll et al, 1982; Deb et al, 1983; Gowal et al, 1984 and Bhan et al, 1987).

This variation in different studies represent true geographical differences, the time of the years of study and different assay employed for heat labile enterotoxin may influence incidence rates (Deb et al, 1983).

Paul et al (1980) reported ETEC in 8.67% of hospitalised children with diarrhoea, but in this study difference in prevalence rates of enterotoxigenic E. coli in children with diarrhoea and controls was not significant statistically. This study was conducted over a limited period of time and over a small number of cases.

Panhotra et al (1980) conducted a year long study in Chandigarh and isolated ETEC in 13.8% children with diarrhoea. In this study a total of 99 strains of E.coli were isolated from 127 children with acute diarrhoea below 2 years of age. Only 13 of them were enterotoxigenic 5 strains showed production of LT/ST, 4 produced only ST and 4 only LT.

Black et al (1980) conducted a study at Matlab Hospital in Bangladesh. Over 6500 stool specimens were examined in 2 years period (They also examined ETEC in diarrhoea in other age groups). ETEC were found to be responsible for 28% of all diarrhoea cases seen at the hospital. The incidence was highest in age group below 2 years.

In a study in rural Bangladesh 197 diarrhoeal children age 2-60 months were studied for its aetiology. The ETEC were isolated most frequently (27%) followed by rotavirus and shigella. 85% of ETEC-ST were associated with the diarrhoea compared with only 62% of ETEC-LT of 70% ST/LT-ETEC were associated with diarrhoeal illness (Black et al, 1981).

Deb et al (1983) studied 201 preschool children suffering from acute diarrhoeal illness and investigated them to determine the prevalence and seasonal occurrence of ETEC diarrhoea in a periurban community near Delhi. 77 age matched children formed the control group. ETEC was isolated from faecal specimen of 22.9% patients and 7.9 in control group ($p < 0.01$). No seasonal fluctuation was demonstrated.

Bhan et al (1987) conducted a study in slum community near Delhi. This included 204 children below 5 years of age and 98 controls. ETEC in this study was present in 23% children with acute diarrhoea. Peak incidence of ETEC was observed during second and third years of life (26.1% and 29.1% respectively).

Bhat et al (1985) reported 7.4% incidence of ETEC diarrhoea in a hospital based study from Bangalore in children under five years of age.

Jaysheela et al (1989) studied the characteristics of 75 E. coli isolates from cases of diarrhoea in infant and children from different parts of the country and

concluded that (i) ETEC is the most important cause of E.coli diarrhoea amongst infants and children in our country followed by EPEC and ETEC (ii) LT producers are more common in our country than LT/ST or ST strains.

CLINICAL FEATURES

The clinical illness caused by ETEC is largely the same whether the strains produce one or both of the enterotoxins, range from mild diarrhoea to severe cholera like disease. Both the enterotoxins produce a secretory diarrhoea in small intestine (WHO Scientific group, 1980).

Black et al (1981) in their study indicated that ETEC diarrhoea last considerably longer in infants than adults. In children below two years of age they observed along with watery diarrhoea in all 58 infants, 53 had vomiting and 30 had fever. The mean duration of diarrhoea was 39.5±3.8 hours before hospitalisation, 61.8±5.5 hours after hospitalisation and total duration was 101.4±6.7 hours.

Asymptomatic infections with ETEC have been well documented in many studies of non hospitalised children (Black and Merson, 1981).

ENTEROPATHOGENIC E.COLI (EPEC)

Diarrhoea associated with faecal E. coli has been recognised for almost a century but the presence of E.coli in normal stools made it hard to distinguish which type of E.coli can cause diarrhoea and which are normal commensals in gastrointestinal tract. A particular serological type

of E. coli was associated with most episode of summer diarrhoea in infants in Britain (Bray, 1945) and this was followed by introduction of serotyping to identify strains of E.coli in outbreaks of diarrhoea. Identification of E. coli by serotyping was used widely and by early 1960's there were 170 known serotypes of E.coli although only a dozen or so were found commonly. When enterotoxins became recognized it was found that most strains of EPEC were enterotoxigenic, since then setotyping became unpopular as a routine diagnostic procedure by late 1970's (Gracey, 1980). EPEC seems to have become relatively unimportant as a cause of diarrhoea in infants and young children in developed countries. But EPEC continue to be important in developing countries.

Strains of EPEC which are non-invasive and which do not produce LT or ST, have been found, which are nevertheless able to cause diarrhoea (Annohymus, 1983). Other toxins might be involved in diarrhoea pathogenesis for example Vero toxin (VT) (Konowalchuk et al, 1977). Attachment of EPEC to intestinal mucosa is an essential virulance mechanism and this, itself may be involved in diarrhoea causation by production of faecal areas of mucosal damage to intestinal brush border at site where EPEC attach to the gut (Clausen and Christie, 1982).

The epidemiology of EPEC disease in developing countries is not so well defined. EPEC in these areas are more frequently isolated from diarrhoea cases in

second six months of life. Institutional outbreak have been less common and community outbreaks are more common (WHO, Scientific group, 1980).

Sanyal (1981) in his review of "epidemiological importance of diarrhoeal agents in India", stated that EPEC constitute most important etiologic agents of diarrhoea in infants and children in India. He observed that amongst commonest serotypes 026 is found in all three region of the country viz. north, south, and the east. The commonest serotypes in north zone 020, 026 and 086 from south zone 0.26 0.55 and 011 and from the east zone are 026, 0127 and 0128. The reported incidence of EPEC diarrhoea from India ranges between 5 to 18% (Sanyal, 1981).

Sarkar et al (1980) studied the role of entero-pathogenic E.coli associated with diarrhoeal disease in children admitted in a hospital in Delhi. Out of a total 1326 patients, 223 strains of E.coli isolated in pure culture were serotyped. A total 58.8% strains could be serotyped. 018, 026, 020, 086 and 0126 were most prevalent sertotype, in that order.

Agarwal et al (1981) studied bacterial aetiology of acute diarrhoea in infants and children below 2 years of age in an out patient clinic in Chandigarh. 127 children with acute diarrhoea were studied. In acute diarrhoeal group E.coli was isolated as predominant growth in 77 (66.66%) of cases, only 10% strains belonged to classical serotypes. Enteropathogenic serotypes of E.coli were recovered from 8% of control cases.

Gupta et al (1985) in a prospective epidemiological study of acute diarrhoea in children below 3 years of age in semiurban slum community observed that E. Coli contributed for 23.59% possible etiological agent. 1/4th of isolated strains were typable with 15 antisera used 0:18 was the commonest serotype.

Sen et al (1985) in hospital based study in Calcutta, in children under five years, implicated EPEC as possible etiological agent in 17.2% cases of acute diarrhoea. EPEC was predominant agent isolated in infants below 6 months.

Bhat et al (1985) in hospital based study from Bangalore found EPEC as etiologic agent in 9.7% cases of acute diarrhoea in admitted patients below 5 years of age.

Bhan et al (1987) reported incidence of EPEC diarrhoea 7.8% of children with acute diarrhoea attending a dispensary.

SHIGELLA

Shigellosis is one of the common causes of diarrhoea or dysentry among young children (Agarwal et al, 1981). Shigellosis is some times known as bacillary dysentry. There are four recognized species of Shigella - S. dysenteriae, S. flexneri, S. boydii and S. Sonnei. These micro-organisms invade the large intestine, some times into the terminal ileum and cause intraepithelial proliferation and inflammation, penetration beyond lamina

propria is rare. Invasiveness can be demonstrated by gunea pig kerato conjuctivitis model (The sereney test) or in the cell culture. Some patients may have predominantly watery diarrhoea, instead of more common typical dysenteric features. The watery diarrhoea is produced by Shigella enterotoxin (which has molecular weight 30,000) and have cytotoxic property. The exact role of this toxin is not known.

Shigella, one of the most important bacterial agent of acute diarrhoea has been studied extensively. The reported incidence of this organism in diarrhoea ranges from 4-39 percent. Shigella has been found to be present in endemic, epidemic and localised outbreaks of acute diarrhoea. Analysis of species and serotype distribution shows S. flexnari to be most prevalent species in India (Sanyal, 1981).

Feldman et al (1970) reported the prevalence of Shigella in 6.7% preschool children in a southern Indian semiurban community. The incidence of infection below 6 months was low (1.3%). The commonest strain identified was S. flexnari followed by S. Sonnei. The percentage of isolation for any Shigella serotype was highest in 1-2 year age group.

Sanyal et al (1977) isolated Shigella in 6.3 percent children with acute diarrhoea. All the 4 strains were prevalent in their study.

Agarwal et al (1981), in their study of bacterial etiology of acute diarrhoea in children below 2 year of age isolated 43.3% bacterial enteropathogen. Shigella constituted 14.9% of the isolated enteropathogen.

Stoll et al (1982) conducted a study at ICDDR Bangladesh in patients attending treatment centre over a period of one year. They isolated Shigella from 11.6% of 3350 patients studied. And it was second most common enteropathogen over the age of 2 years. The clinical manifestation found were :-

1. Watery diarrhoea occurring in young children and associated with shorter duration of illness and with more vomiting and dehydration.
2. Dysentry with stool blood and abdominal pain were the most useful sign and symptoms for diagnosis of shigellosis. Simple visual inspection of stool blood was correctly identified in 44% of all the cases infected by shigella

Santhanakrishanan et al (1987) studied the clinical spectrum of disease in children under 5 years of age. Among 250 stool samples, examined Shigella species were isolated from 22% cases. Fever (69.09% cases) Vomiting (71%), Tenesmus and rectal prolapse (50%) and dehydration were presenting features. Children under 2 years of age were predominant victims and 80% of cases identified were under two years of age. Measles and malnutrition played a greater role in predisposition to diarrhoea and dysentry.

Associated infections like pneumonia and septicemia are significant contributory factors for increased morbidity and mortality among children with shigelosis (Alam et al, 1984). Hemolytic uremic syndrome with acute renal failure has been an important complication of shigellosis reported from Sri Lanka(Lambadsuriya, 1986).

Mohandas et al (1987) in their etiological study of patient (⁄3 years of age) attending an out-patients clinic in Vellore, identified shigella in 15% cases. Out of total 245 children studied 36 had Shigella infection. Mucous, blood in stool was present in 12, 8 had watery diarrhoea, half of the children had fever, 19 had abdominal cramps. The syndrome of classical dysentry was associated (p ⁄0.001) with shigellosis. It was also observed that infants younger than 6 months, breast fed children were unlikely to have shigella infection. Fever and presence of mucous in stools was not significantly associated with shigella infection in this study.

Dutta et al (1989) studied the clinical and bacteriological profile in shigellosis in Calcutta before and after an epidemic (1984-1987) in children (below 5 years of age) hospitalized for shigellosis over a period of 4 years. During 1984 epidemic of shigellosis - it was isolated from 46.6% of dysentry patients and from 22.8% patients of watery diarrhoea. The predominant serotype observed was S. dysenteriae type I from dysentry(37.3%)

and watery diarrhoea patients (17.1%). During post epidemic period in 1985 and 87, 27.9% and 8.3% Shigella were isolated from dysentry and watery diarrhoea patients respectively. Then the isolation rate of Shigella flexnery increased

Shigella infected patients presented with both syndrome of dysentry and watery diarrhoea. Vomiting was more common with the watery diarrhoea while fever was seen in both patients with dysentry and watery diarrhoea.

CAMPYLOBACTER JEJUNI

Campylobacter jejuni (formerly referred to as a related vibrio) is a relatively recent addition to the growing list of agents causing infective diarrhoea. In several developed countries, the frequency of isolation of this organisms from patients of acute diarrhoea has transcended that of conventional enteropathogens like shigella and Salmonella . In recent years the significance of C. jejuni as an etiologic agent of human enteric disease has been established in developing countries. However, the detection of large number of inapparent infections particularly among infants and children in developing countries makes it difficult to assess the extent of the problem of campylobacteriosis.

The organism has been isolated from 4 to 15% of children with acute diarrhoea in both developed(Butzler et al, 1973 and Pai et al, 1979) and underdeveloped

(Stoll et al, 1982 and Blaser et al, 1980) countries. From developed countties the organism was rarely isolated from healthy individuals.

Nayyar et al (1983) studied the prevalence of C. jejuni diarrhoea among 155 children suffering from acute diarrhoeal illness. C. jejuni was isolated from 16(10.3%) of the patients and 2(4.7%) of the controls ($p > 0.05$). The difference in excretion rate of C. jejuni from patients with diarrhoea and controls was insignificant in this study. The highest frequency of isolation was in 61-144 months age group. The organisms was not isolated from any child below 5 years of age. The clinical features consisted of fever (68.8%), abdominal pain (43.8%) blood with stools (43.8%), the usual duration of diarrhoea was more than 1 week.

Rajan et al (1982) reported 14.8% isolation of C. jejuni from healtny children in a random sample from southern India.

In another study from Calcutta (Nair et al,1984), C. jejuni was isolated from 7.7% of 392 hospitalised cases with acute diarrhoea. The recovery rate from normal healthy individuals was 4.4%. The preschool children were most commonly affected. In this study the high incidence of mixed infection was observed with other known enteropathogens. Khatua et al (1984), in their study, isolated C. jejuni in 2% cases and none from asymptomatic controls.

The clinical features of patients with C. jejuni appears to be quite different from that encountered in the developed world. The majority of the patients excreting C. jejuni as the sole pathogen had watery diarrhoea (Similarly in Bangladesh, the complaints of all diarrhoeal patients versus those with C. jejuni infection were identical with two exception : abdominal pain was less common among patients with C. jejuni and watery diarrhoea was slightly more common.

NON TYPHOID SALMONELLAE

Salmonellae is well known organisms in causation of acute diarrhoea. It is commonest in children under age of 5 years and particularly in infants under 12 months of age. The disease can be spread through contaminated food (such as poultry) milk or water and perhaps requires a relatively large inoculum. A wide range of species can affect human, and all serotypes of salmonella except S. typhi, and S. paratyphi A, can cause gastroenteritis in man and animals. The incidence of gastroenteritis caused by different serotype varies from time to time and place to place (WHO, 1980).

Diarrhoea initiated by Salmonella may be produced by several mechanisms. Many patients present with non-specific watery diarrhoea, clinically identical to that caused by enterotoxigenic E. coli, several toxins have been identified but whether they are responsible for excess

intestinal fluid production in human remains to be proved. Salmonella can also initiate diarrhoea by indirect stimulation of energy system within epithelial cells. Salmonella can penetrate superficial layer of mucosal lining without destroying epithelial cells and sometimes Salmonella infection can disseminated.

The incubation period of the salmonella diarrhoea is short, 8 to 48 hours. It is characterised by nausea, vomiting, abdominal pain, loose watery diarrhoea, which sometimes may contain mucous and blood. Vomiting is usually not severe fever 101°F to 102°F is seen in as many as 70% of patients. Septicemia to Salmonella is more common to first three months of life (Fiegin, 1987).

Salmonella is a causative agent of diarrhoea in India have remained relatively of less importance due to food habit even though strains are isolated in community based studies and also in outbreaks (Sanyal, 1981). The incidence of Salmonella in acute diarrhoea varies from 0.10% in different studies. (Sanyal et al (1977) failed to isolate Salmonella species in the study of 206 diarrhoeal children. Bhan et al (1987) isolated 2.5% Salmonella in their study. Similarly Sen et al (1985), Gupta et al (1985) have reported relatively lower incidence of Salmonella (0.9%, 1%) respectively). Bhat et al (1985) from Southern India have reported 10.8% prevalence rate of Salmonella in causation of acute diarrhoea. Fule and Kaundinya (1985) reported incidence of isolation to be

4.91% from a rural hospital of Maharashtra, in this study Salmonella typhymurium was the commonest strain(80%). Ram et al (1987) detected in Salmonella 10%(178 positive patients out of total 1980 patients) with diarrhoea attending a hospital, S. typhimurium and S. senftenberg comprised the bulk of serotype isolated. The incidence of diarrhoea due to Salmonella was maximum amongst the patient below one year of age and it decreased gradually with increase of the age.

Although Salmonella seems to constitute 2-5% cases of diarrhoea, they have potentiality of causing severe, possibly lethal illness.

ACUTE DIARRHOEA IN BREAST FED VS NONBREAST FED

Breast milk provides not only nutrition for the growing infants but also active protection against the development of infections, particularly those of gestro-intestinal tract. Apart from being hygeinic, largely uncontaminated, breast milk is also endowed with many protective substances such as immunoglobulins, lymphocytes, macrophages, lysozymes and lacto-ferritin which may play an anti-infective role particularly in the gut (Goldman and Smith, 1973).

Educated and afluent section of the community living under conditions of good hygiene can bottle fed their babies safely (Clavano, 1982). Most mothers in

developing countries have neither the knowledge nor the money, time, sanitory conditions, nor the basic facilities to bottle fed their babies. For many of these mothers feeding bottle is indeed a baby killer (Muller, 1975). Lack of hygiene and education allows the feeding bottles to be heavily contaminated with bacteria and causing the baby to have frequent episodes of diarrhoea.

Studies from the developing countries have unequivocally shown the protective role of breast feeding in infectious disease (Mata et al, 1976 and Kumar et al, 1981). Mata et al (1976) in their prospective study found that adequate growth and survival were characteristics of exclusively breast fed infants in first months of the life. Despite high rate of infection, children exhibited considerable resistance to intestinal protozoa, enterobacteriacae and enteric viruses. Resistance against colonic invaders is attributed to bifidus flora and that against agents acting in small bowel. In this study diarrhoeal disease was least during breast feeding period and increase with the weaning to reach maximum peaks at the time of weaning.

Kumar et al (1981) in their prospective study carried out from birth to one year of age on under privileged rural Indian infants and privileged urban to study the effect of feeding pattern on morbidity and mortality. The lower mortality ($p < 0.01$) and morbidity ($p < 0.01$) were recorded in breast fed babies. Irrespective of place of

residence, socio-economic status and maternal education. Diarrhoea was less common ($p < 0.001$) among the breast fed infants. More so during the first four months of the life, in this study difference in morbidity becomes less marked during five to twelve months. As almost all of the breast fed babies were given some supplements and in this group maximum number of morbidity were noted. Incidence of diarrhoeal disease in mixed fed and bottle fed babies were significantly higher.

Saran et al (1979) from Varanasi found the higher prevalence of diarrhoeal illness in breast fed infants as compared to mixed fed infants and children. Bhatia et al (1980) also found that incidence of gastroenteritis is definitely lower in breast fed infants.

Many studies from more affluent countries in recent years have shown either a moderately decreased incidence in diarrhoeal disease and other infections or no significant difference.

Mittal et al (1983) in their hospital based study found that prevalence of breast fed infant among the non-diarrhoeal infants was significantly ($p < 0.01$) higher than in diarrhoeal infants. In the same study microbial flora of the gut was studied in relation to feeding pattern. No differences were observed in frequency of various organisms isolated from rectal swab of breast and bottle fed infants with or without diarrhoea. Highly pathogenic organisms EPEC, ETEC, Shigella and Salmonella were

frequently cultured from exclusively breast fed infants with diarrhoea. This study suggested that the breast feeding in the environmental conditions prevailing in communities is rather inadequate to protect against the development of diarrhoeal disease with highly pathogenic organisms.

Fallot et al (1980) however, failed to find any bacterial pathogen among hospitalised diarrhoeal infants who had been largely breast fed. Cushing and Anderson (1982) failed to find any protection against toxigenic E. Coli amongst breast fed infants. Weinberg et al (1984) observed that the incidence of Rotavirus infection, its average age of occurrence and severity among differently fed infants were largely comparable. The protection against rotavirus infection is only marginal in breast fed.

It is noteworthy that the diarrhoea morbidity and mortality continues to be significantly prevalent in areas of the world where the breast feeding is the dominant mode of feeding. Protective effects of breast feeding are more obvious in poor socio-economic areas with heavy environmental contamination. The protective effects observed even in these areas are also not absolute even among purely breast fed infants. The breast feeding can provide a significant but not absolute protection against diarrhoeal disease (Mittal, 1986).

ACUTE DIARRHOEA AND MALNUTRITION

Acute diarrhoea and malnutrition are commonly associated in many of the developing countries. Diarrhoea may affect nutritional status in several ways.

1. The intake of food due to anorexia is decreased.
2. Loss of macronutrients and micronutrients in the faeces (Einestein et al, 1972).
3. Decreased diarrhoeal gut enzyme activity.
4. Catabolic response to infection (Biesel, 1975).
5. Withholding the food as a measure to control diarrhoea.

Diarrhoeal disease is so devastating to infants (Synder and Merson, 1982) in regions of world where malnutrition is prevalent. It is suspected that somehow undernourished child may be particularly vulnerable to infectious diarrhoea. There are some clinical observations to suggest that acute diarrhoeal disease is more frequent and more serious among the malnourished subjects than among those of normal nutritional status (Hansen et al, 1962; Robertson et al, 1960 and Ghai and Jaishwal, 1970).

Based on a two year longitudinal study of diarrhoea in infancy and early childhood Ghai and Jaiswal (1970) reported spell frequency of diarrhoeal disease during the phase of undernutrition. In this study 60% undernourished children suffered from diarrhoea disease as compared to 20% of those with normal nutritional status. When children with diarrhoeal disease in preceding period of three months or more deteriorated nutritionally due to

dietary factors, the spell frequency of diarrhoea was 8.2 per 100 child weeks, during succeding period of observations as compared to frequency of 3 episodes per 100 child weeks in children maintaining the normal nutritional status. Improvement in nutritional status likewise reduced the spell frequency.

Recent studies from NIN Hyderabad and Bangladesh have failed to show any increase in the attack rate of diarrhoeal illness. Chen et al (1981) on the basis of a prospective field study in Bangladesh reported that child nutrition appears to exert little effect on subsequent diarrhoeal incidence. This study suggested that impact of diarrhoea in predisposing and exacerbating malnutrition may be the most important of the bidirectional interaction. The predominant effect of nutrition on diarrhoea may be through disease incidence. Palmer et al (1976) demonstrated that duration of cholera purging among malnourished children was greater than among well nourished children, presumably because turnover and replacement of diseased gut epithelial cells were decreased due to malnutrition.

Mittal et al (1980) studied the clinical, biochemical and bacteriological profile of acute diarrhoea in malnourished children. In their study severe dehydration was more often seen in grade III malnutrition children. Serum sodium levels were significantly lower in malnourished children but it had no effect on outcome. On the other hand hypokalemia in malnourished children was more often fatal.

Bacteriological flora was significantly different in these two groups. Pathogens like Salmonella, Shigella and Klebsiella were more often grown in malnourished group as compared to well nourished group.

Malnourished children with already compromised nutritional status could be expected to tolerate diarrhoea poorly. Chen et al (1980) have shown as increase of 3 to 4 times in diarrhoeal mortality among malnourished children compared to that in normal nourished children. Mittal et al (1980) observed a mortality rate of 10.5% among malnourished children as compared to 2.6% in normal nourished children. In their study fluid loss and electrolyte imbalance was the major cause of the morbidity and mortality in these cases.

Ray et al (1986) studied the specific enteropathogens in relation to severe malnutrition in children with acute gastroenteritis. Enteropathogenic profile was studied in 65 malnourished children and 50 well nourished children with acute diarrhoea. Pathogenic organisms like Salmonella and Shigella were isolated in significantly higher number in malnourished children with diarrhoea as compared to well nourished children ($p < 0.001$) while rotavirus detection was greater in well nourished children.

The relationship between malnutrition and infection are synergistic and each factor adversely

affects the other. Protein energy malnutrition has been identified as most frequent cause of acquired immunodeficiency in man (Seth, 1985). There may be general increased susceptibility to conventional entrapathogens and associated malnutrition may adversely affect mucosal and enzymatic functions.

MATERIAL AND METHODS

MATERIAL AND METHODS

This study was carried out in the department of Paediatrics and Microbiology, M.L.B. Medical College Hospital, Jhansi. A total of 189 children in the age group of 1 to 60 months with acute diarrhoea were studied. Cases were selected from Paediatrics out patient clinic and Paediatric ward.

One hundred age and sex matched non-diarrhoeal controls were selected from admitted patients for minor illnesses like Asthmatic bronchitis, epilepsy, etc. and from asymptomatic siblings of admitted patients, with no history of diarrhoea in last 15 days.

A detailed history was recorded and clinical assessment and microbiological investigations were carried out as detailed below.

HISTORY

A detailed history was taken from mother or a family member of the patient in particular for duration of illness, quantity, frequency, and consistency of stools, Duration and frequency of vomiting, presence of fever, pain in abdomen, tenesmus, duration of period when last urine is passed, for any complication like seizure, abdominal distension, anuria etc. A detailed history was recorded from mother regarding pattern of feeding. Whether baby is bottle fed, breast fed or mixed fed (bottle + breast), or fed by cup and spoon. Attempt was

also made to judge the standard of bottle hygeine by questioning the mother, and details of medication and fluid therapy taken earlier was also recorded.

PHYSICAL EXAMINATION

A detailed physical examination was carried out This included a preliminary general examination. The degree of dehydration was assessed according to well known criteria of WHO. Apart from this child was also examined for presence of fever, URI, oral thrusth, perianal excriation or depigmentation for any septic focus. The routine systemic examination was carried out.

Assessment of nutritional status was done according to classification of Indian Academy of Paediatrics.

COLLECTION OF SAMPLE

Rubber capped clean glass vials were supplied to the mothers so that they could collect a part of stool sample of their children in the morning just after defaecation. The vial with specimen was labelled and was brought to the laboratory within 2 hours.

MACROSCOPIC EXAMINATION

The sample was examined for the consistency of the stool, presence of blood, mucous and adult parasites by naked eye.

MICROSCOPIC EXAMINATION

Direct microscopy was done in normal saline preparation and Lugol's iodine preparation. On a clean glass slide 2 drops of normal saline on side and 2 drops of Lugol's iodine on the other side were placed. With a match stick a small portion of faeces (about 2 mg) was picked up and emulsified in each of the drops. The smears were covered with cover slips and examined for ova, trophozoites and cysts under low (100 x) and high (400 x) magnification. Erythrocytes and pus cells were also looked for.

CULTURE

All the specimens were plated on Mac Conkey agar (MA), deoxycholate citrate agar (DCA) and thiosulphate citrate bile salt sucrose (TCBS) agar plates. The plates were incubated at 37°C over night. MA and DCA plates were examined for lactose fermenting (LF) and lactose non-fermenting (NLF) colonies. LF colonies of pure and predominant type (more than 80% colonies looked alike) and all NLF colonies were processed. TCBS agar plates were looked for flat yellow colonies of vibrio cholerae and green mucoid colonies of V. parahaemolyticus. One suspected colony was picked up and inoculated in 2 ml peptone water. After 3-4 hours of incubation in peptone water each culture was inoculated with a straight wire into triple sugar iron agar (TSI), sulphide indole motility medium (SIM), Christensen's urea, Simmon's citrate, Falkow's decarboxylase broths and

peptone water sugars, like glucose, sucrose, mannitol and lactose. Oxidase and catalase tests were also performed. All the strains were identified following Cowan and Steel (1974). The strains were confirmed by agglutination test with specific antisera.

For isolation of Campylobacter, Blaser's medium was used. After inoculation, the plates were incubated at 42°C in a candle jar for 48 hours. After incubation, the plates were looked for either small discreet greyish colonies or irregular, watery colonies running along the wire track. The suspected colonies were confirmed by Grams' stain, motility, catalase, oxidase hippurate hydrolysis and no growth at 25°C and in 3.5% NaCl.

DETECTION OF ROTAVIRUS

On receipt of sample in the laboratory the procedure of preparation of faecal extract and detection of rotavirus antigens in them by the ELISA technique was according to the manual provided with the kit of reagents (The ELISA kit was from WHO collaborating centre on Human Rotaviruses, Birmingham Hospital, Birmingham, U.K.).

About 1 gram of faeces was taken in a centrifuge tube and emulsified in 10 ml of sterile phosphate buffered saline (PBS) at pH 7.3. The emulsion was centrifuged by low speed centrifugation i.e. approximately 2000-3000 g for 10-15 min. After centrifugation the supernatant (which is 1/10 dilution of faecal extract) is collected in

screw capped bottle and stored at -20°C till sufficient number of samples had accumulated.

The procedure of the screening ELISA test is briefly as follows. A flat bottomed micro-titre plate (NUNC) was coated with 0.1 ml of 1 : 10000 of rabbit antirotavirus serum at 4°C overnight. Then the plate was washed six times with phosphate buffer saline with Tween 20 (PBS/T). The test sample was then added (0.025 ml + 0.075 ml of buffer) to duplicate wells and incubated at 4°C overnight. Known positive (near and 1/10 diluted) and negative controls were included in addition to substrate and conjugate controls. The plate was then washed six times in PBS/T as before. Then 0.1 ml of 1 : 10000 guinea pig anti-rotavirus serum was added to each well except substrate and conjugate controls. The plate was sealed and incubated at 37°C for 2½ hours. The plate was washed again and 0.1 ml of 1 : 800 goat anti-guinea pig antibody labelled with alkaline phosphatase (conjugate) was added. After incubation at 37°C for 1½ hour and washing 0.1 ml of substrate containing 1 mg/ml of p-nitrophenyl phosphate was added to each well. After 10-20 minutes, when the diluted positive control wells had developed a yellow colour, the reaction was arrested by addition of 0.05 ml of 3N sodium hydroxide. The results were read by naked eyes.

O B S E R V A T I O N S

OBSERVATIONS

One hundred and eighty nine children suffering from acute diarrhoea under the age of five years were studied. One hundred non-diarrhoeal children were parallely studied as controls. Infants below one month of age were not included in this study.

TABLE I : Age wise distribution of study and control cases.

Age groups (months)	Study cases		Control cases	
	No.	Percentage	No.	Percentage
≤6	34	18.0	19	19.0
7 - 12	40	21.0	23	23.0
13 - 24	57	30.1	30	30.0
25 - 36	30	15.9	16	16.0
37 - 60	28	14.8	12	12.0
TOTAL	189	100.0	100	100.0

The male : female ratio in study group was 1.4:1 and in control group it was 1.5 : 1. In the study group 39% cases were below one year of age, 69.1% below two years of age and 31% cases were between 2 to 5 years of age. In the control group, age distribution of cases was almost similar to that of study group (Table I). As such the children in both the groups were comparable in respect of age and sex.

Microbiological study was carried out in all the 289 children. The various organisms detected in stool examination are depicted in table II.

TABLE II : Microbiological findings of stool examination.

Pathogens isolated	Study cases (N=189)		Control cases (N=100)	
	No.	Percentage	No.	Percentage
Rotavirus	34*	18.0	1*	1.0
+E. Coli	41**	21.7	11**	11.0
Shigella	15	7.9	-	-
Salmonella (nontyphoid)	3	1.6	-	-
Campylobacter jejuni	11***	5.8	4***	4.0
Pseudomonas	2	1.1	-	-
Klebsiella	2	1.1	-	-
Vibrio-cholerae	-	-	-	-
E. Histolytica	5	2.6	-	-
G. lambilia	6	3.2	-	- -
Mixed	4	2.1	1	1.0
TOTAL	123	65.1	17	17.0

* $p < 0.001$, ** $p < 0.05$, *** $p > 0.05$

\+ E.Coli strains as pure or predominant growth on culture. E.coli strains could not be screened for EPEC and ETEC.

Rotavirus was demonstrated using ELISA technique in 34 out of 189 children (18%) in study group and 1 out of 100 in controls (1%). Bacteria belonging to 6 species were isolated from 40.7% cases. The most commonly

isolated bacteria was E.coli (21.7%) from the study group and 11% from controls. , Isolates of E.coli were not subjected to serotyping and tests for enterotoxigenecity Vibrio cholerae and V. parahaemolyticus could not be isolated from any case or control. Shigella was isolated in 7.9% cases and none from controls. Salmonella was isolated from 3 cases. Pseudomonas and Klebsiella were isolated from two cases.

Campylobacter jejuni was found in 11 out of 189 diarrhoeal children (5.8%) and 4 out of 100 non-diarrhoeal children ($p > 0.05$). E. histolytica and G. lamblia were isolated from 2.6% and 3.2% diarrhoeal cases respectively and nonefrom controls. Mixed pathogens were isolated from 4 cases. Amongst 4 mixed pathogens, E. coli + campylobacter were present in 1 case, E.coli + Shigella were present in one case and E. coli + Rotavirus were present in two cases.

Detection rate of rotavirus was highest(35%) in children of 7-12 months of age. No rotavirus was detected after 3 years of age in the present study. Below age of 6 months, rotavirus was detected in 17.5% cases. Isolation rate of E. coli was higher during infancy. Shigella was found to be more common after infancy. Out of 11 campylobacter isolates 8 were present during 7-24 months of age. Out of 3 Salmonella isolates 2 were present in children below 6 months of age. G. lamblia and E. Histolytica were not isolated in infants in present study.

TABLE III : Frequency of isolation of various aetiological agents in relation to age.

Pathogens	Age groups (months)					
	≤ 6 (n=34)	7-12 (n=40)	13-24 (n=57)	25-36 (n=30)	37-60 (n=28)	Total (n=189)
Rotavirus	6(17.5)	14(35.0)	12(21.0)	2(6.7)	-	34(18.0)
E. Coli	8(23.5)	10(25.0)	13(22.9)	3(10.0)	7(25.0)	41(21.7)
Shigella	1(2.9)	2(5.0)	5(8.8)	4(13.3)	3(10.7)	15(7.9)
Salmonella	2(5.9)	-	1(1.7)	-	-	3(1.6)
Campylobacter	1(2.9)	3(7.5)	5(8.8)	1(3.3)	1(3.6)	11(5.8)
Pseudomonas	1(2.9)	-	-	1(3.3)	-	2(1.1)
Klebsiella	-	-	1(1.8)	1(3.3)	-	2(1.1)
E. histolytica	-	-	2(3.5)	1(3.3)	2(7.1)	5(2.7)
G. Lamblia	-	-	1(1.8)	3(10.0)	2(7.1)	6(3.2)
Mixed agents	2(5.9)	-	-	1(3.3)	1(3.6)	4(2.1)

TABLE IV : Feeding pattern in study and control cases.

Mode of feeding	Diarrhoea cases Age groups (months)				Non-diarrhoeal cases Age groups (months)			
	< 6	7-12	13-24	Total	< 6	7-12	23-24	Total
Exclusively breast fed	19* (55.9)	14 (35.0)	9 (15.8)	42** (32.1)	16* (84.2)	9 (39.1)	9 (20.0)	31** (43..1)
Bottle + breast fed	12 (35.3)	18 (45.0)	31 (54.4)	61 (46.6)	2 (10.5)	8 (34.0)	11 (36.7)	21 (29.2)
Exclusively Bottle fed	3 (8.8)	6 (15.0)	6 (10.5)	15 (11.4)	1 (5.3)	4 (17.4)	6 (20.0)	11 (15.3)
Cup and Spoon fed	-	2 (5.0)	11 (19.3)	13 (9.9)	-	2 (8.7)	7 (23.3)	9 (12.5)
TOTAL	34	40	57	131 (100.0)	19	23	30	72 (100.0)

* p < 0.01, ** p > 0.05

Isolation rate of micro-organisms from stool specimens was highest during 7-12 months of age and minimum during 37-60 months of age (Table III).

Feeding pattern of 131 diarrhoeal children and 72 non-diarrhoeal children below 24 months of age is depicted in table IV.

With the increasing age, as expected, the number of children who were exclusively breast fed decreased. But a substantial number of them are mixed fed (Bottle and breast fed). Lesser number of children were fed by spoon and cup (9.9% and 12.5% in diarrhoeal and non diarrhoeal cases respectively).

This table also shows that apparently larger number of children were breast fed in non-diarrhoeal group as compared to diarrhoeal group, but this difference is not statistically significant ($p > 0.05$). However, below 6 months of age exclusively breast fed babies are statistically higher in non-diarrhoeal group ($p < 0.01$).

The prevalence of bottle feeding (exclusively or partly) was present in 58% in diarrhoeal children as compared to 44.5% in non diarrhoeal children.

Bottle hygiene was poor in majority of bottle fed babies (94%).

The organisms isolated from diarrhoeal children with different feeding pattern i.e. breast fed and bottle fed, partly or solely, are shown in table V.

TABLE V : Correlation between aetiological agents and feeding patterns.

Organisms	Exclusively breast fed (n=42)	Bottle + breast fed (n=76
Rotavirus	11(26.2)*	17(22.4)*
E. Coli	10(24.4) *	21(27.6) *
Shigella	2(4.8)*	6(7.9) *
Salmonella	1(2.4) *	2(2.63) *
Campylobacter	1(2.4) *	5(6.5) *
Klebsiella	-	1(1.3)
Pseudomonas	-	1(1.3)
Giardia	1(2.4)	-
E. histolytica	1(2.4)	1(1.3)

p 70.05

It is apparent from the table V that almost identical organisms were detected in both the groups. Rotavirus was detected in 26.2% and 22.4% cases in breast fed and bottle fed children respectively. Prevalence of shigella was apparently higher in bottle fed children as compared to breast fed children, similarly Campylobacter was detected more frequently in bottle fed cases. But these differences were not statistically significant. Klebsiella and Pseudomonas were present in one bottle fed and none in breast fed cases.

TABLE VI : Nutritional status in study group cases.

Nutritional status	Age groups (years)			Total
	< 1	1-3	3-5	
Normal	29	23	10	62(32.8)
Grade I	16	19	6	41(21.7)
Grade II	16	27	7	50(26.5)
Grade III	11	16	4	31(16.4)
Grade IV	2	2	1	5(2.6)
TOTAL	74	87	28	189(100)

Note : Grading of nutritional status according to classification of Indian Academy of Pediatrics.

Table VI depicts that there is preponderance of undernourished children in study group cases. 67.2% children were below 80% of the expected reference weight for the age.

Table VII depicts various micro-organisms isolated in stools examination according to nutritional status. In normal nourished children an aetiological agent could be identified in 56.4% cases, while in mild (grade I and II) and severe malnutrition (grade III and IV) over isolation rates were 65.9% and 68.6% respectively. Rotavirus and E. Coli were isolated in almost equal frequency in all the three groups. Shigella was isolated in apparently larger number of cases in severely malnoushed group but the difference in normal nourished and

severely malnourished children was statistically insignificant.

TABLE VII : Micro-organisms according to nutritional status.

Micro-organisms	Nutritional Status		
	Normal (n=62)	Grade I+II (n=91)	Grade III+IV (n=35)
Rotavirus	12 (19.4)	16 (20.9)	6 (17.1)
E. Coli	13 (21.0)	21 (23.1)	8 (22.9)
Shigella	5 (8.1)	6 (6.6)	4 (11.4)
Salmonella	-	2 (2.2)	1 (2.9)
Campylobacter	3 (4.8)	6 (6.6)	2 (5.7)
Klebsiella	-	1 (1.1)	1 (2.9)
Pseudomonas	-	2 (2.2)	-
E. histolytica	1 (1.6)	3 (3.3)	1 (2.9)
Giardia Lamblia	1 (1.6)	3 (3.3)	2 (5.7)
TOTAL	35 (56.4)	60 (65.9)	24 (68.6)

Figures in parantheses are percentage.

Dehydration is the mosti important consequence of acute diarrhoea. Out of 189 patients who were selected for study from out patient clinic, 102(54.0%) had no dehydration, 42(22.2%) had mild, 28(14.8%) had moderate and 17(9.0%) had severe dehydration. 56 children were admitted in Paediatric ward for management. Acidosis was present in 12, convulsions in 3 and abdominal distension in 5 cases among 56 admitted cases. The admitted cases were managed with intravenous fluid and oral rehydration according to usual protocol. All the admitted cases recovered uneventfully.

Thirty four micro-organisms were isolated from these 56 cases. Rotavirus was present in 11 cases. E.coli in 15, Shigella in 5 and Salmonella and Campylobacter in 2 cases each.

Minor associated problems like oral thrust, and perianal excariation were present in 9 and 7 cases respectively. Prolapse rectum was observed in 11 cases out of these, 3 cases were Shigella positive.

Out of total 189 children, 92(48.7%) presented with watery diarrhoea, dysentry defined as presence of blood in stool with or without mucous was present in 26 (13.8%). 74(39.2%) patients had vomiting. Pain in abdomen was present in 22.2% cases. Fever was present in 77(40.7%) and upper respiratory tract infection in 74(39.1%).

Clinical features of diarrhoea due to various micro-organisms is presented in table VIII. In rotavirus

TABLE VIII : Clinical features and some aetiological agents.

Clinical features	Rotavirus (n=34)	E. Coli (n=41)	Shigella (n=15)	Campylobacter (n=11)	No pathogen (n=66)
Stool frequency per day	8±5.1	8.2±4.1	10.4±5.7	8.8±2.5	7.6±4.3
Fever	18(52.9)	14(34.1)	10(66.7)***	5(45.5)	21(31.8)
U.R.I.	18(52.9)	18(43.9)	4(26.7)	5(45.5)	24(36.4)
Vomiting	24(70.6)*	15(36.6)	3(20.0)	3(27.3)	21(31.8)
Watery diarrhoea	28(82.3)*	19(41.5)	4(26.7)	6(54.5)	32(48.6)
Blood in stools	-	4(12.2)	10(66.7)*	3(27.3)	8(12.1)
Mucoid stools	6(17.6)	8(19.5)	17(73.4)*	4(36.4)	17(25.8)
Pain in abdomen	2(5.9)	11(26.8)	7(46.6)**	4(36.4)	14(21.2)

* chi squire test $p < 0.001$, *** chi squire test $p < 0.05$

** $p < 0.01$

Note : Significance of the difference of the frequency of symptom or sign in the children with indicated aetiology and in children with other aetiology.

infected cases, diarrhoea was mainly watery. Out of 34 rotavirus positive cases 28(82.3%) had watery diarrhoea, rest had semiformed or semiliquid stools. Vomiting was present in 24 (70.6%) patients. None had blood in stools. However, 6 children had mucoid stools. Fever and upper respiratory tract infection were present in 18(52.9%) and 18(52.9%) children respectively. Frequency of loose motions were 8.5±5.1 per day.

In E. coli diarrhoea 41.5% had watery diarrhoea. Blood was present in 12.2%, vomiting was present in 36.6% patients. In cases where Shigella was isolated, stools with visible blood with or without mucous was present in 53.3%, mucoid stools were present in 60%. Vomiting was not common (20%). A higher proportion of children had fever (66.7%) and pain in abdomen in 46.0 %.

In diarrhoea due to Campylobacter jejuni, stools were watery in 54.5% cases visible blood was present in 27.3% and 36.4% had mucoid stools. Pain in abdomen was also relatively common (36.4%). In cases where no pathogens were isolated nearly half had watery diarrhoea.

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D I S C U S S I O N

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D I S C U S S I O N

Acute diarrhoeal diseases are an acknowledged major health problem, severely affecting the children from developing counteis, but important to all countries of the world. This disease is perhaps the biggest child killer in developing countries, still 3.5 million children mainly in developing countries die every year due to diarrhoeal diseases. In India alone about 1 million children die of diarrnoeal disease every year.

In several developing countries 40 percent of hospital admissions are accounted for by acute diarrnoeal diseases, with a case fatality rate of 2.5 to 9.5 percent (Singh, 1983). In our pediatric out patient clinic acute diarrhoea constitute nearly 22% of the total attendance. In last one year (June,90 to May,91) 726 patients out of total 2995 admitted patients were suffering from acute diarrhoea, constituting 24.2% of total pediatric admissions. Out of 726 admitted patients 43 patients died(5.92%). Thus, acute diarrhoeal diseases constitute considerable disease burden in hospitals.

Most cases of acute diarrhoea in young children are due to infections with a variety of organisms. The recent recognition of role of certain viruses and bacteria now makes it possible to identiry the causative agents in over two thirds of diarrhoea patients.

The yield of pathogens in stool specimens depend upon the facilities for isolation and identification of micro-organisms. There are a large number of published studies on aetiological agents of acute diarrhoea in children in India, but a small number of these include almost all recently recognised pathogens.

Various Indian investigators, who have included bacterial, viral and parasitic agents especially rotavirus and enterotoxigenic E. Coli in their study have reported 55-80 percent isolation rates of various enteropathogens (Bhat et al, 1985 - 72.3%, Sen et al, 1985 - 81.4%, and 62.1%, Bhan et al, 1987 - 72.3%). Mohandas et al (1987) isolated at least one enteropathogen in 55% cases of acute diarrhoea, In this study authors included investigation for other viruses also but did not include identification of enterotoxigenic E. Coli. Stoll et al (1982) in an extensive study in Bangladesh isolated at least one enteropathogen in 66% of their patients attending a hospital . In the present study overall isolation rate of micro-organisms was 65.2%. But due to non-availability of antisera for serotyping and limitation of facilities, tests of enterotoxigenicity were not carried out.

Rotavirus is the best known viral agent causing acute gastroenteritis. Rotavirus diarrhoea is a significant source of infant and childhood morbidity in tropical and non-tropical settings (WHO, 1989). The importance of rotaviruses in pediatric gastroenteritis has been well

documented in developed countries (Davidson et al, 1974, Kapiakian, 1976; Rodreguez et al, 1977; Brandt et al, 1984 and Ellis et al, 1984 etc.).

In the present study rotavirus was detected in 18% children suffering from acute diarrhoea using ELISA technique. The results obtained in the present study are in agreement with many studies conducted in India. Samantray et al (1982) and Bhan et al (1987) reported prevalence of rotavirus in an urban slum community in to be 21.2% and 20.3% respectively. Mohandas et al (1987) in their study in an outpatient clinic in Vellore observed 19% acute diarrhoeas were due to rotavirus. Bhat et al (1985) and Sen et al (1985) have reported similar incidence of rotavirus in hospitalized children (18.3%, 15.9% and 16.3% respectively). Various authors have reported higher detection rates of rotavirus in patients with acute diarrhoea requiring hospitalisation (Black et al, 1981; Samantray et al, 1982). This is because rotavirus disease has not greater potential to cause dehydration (Black et al, 1981).

Rotavirus diarrhoea is predominantly a disease of children under two years of age (Rodreguez et al, 1977; Black et al, 1982; Samantray et al, 1982 and Brandt et al, 1983). In the present study 32 out of 34 rotavirus positive patients were under 2 years of age, no rotavirus was detected after 3 years of age. The peak incidence of

rotavirus diarrhoea was during 7-12 month. Thus age distribution of rotavirus in the present study is consistent with previous studies. Antibody surveys from many areas of world have also revealed that most children acquire antibody against rotavirus by the end of third year of life (Kapikian, 1975, Jesudoss et al, 1978).

In the present study Shigella was isolated from 15 patients out of 189 patients studied giving a prevalence of 7.9%. Shigella is a constantly occurring organisms in most of the studies. The isolation rate of Shigella in the present study was consistent with the findings of Feldman et al (1970), Sanyal et al (1977), Agarwal et al (1981), Gupta et al (1985). However, isolation rates of Shigella in the present study are less in comparison to studies by Bhat et al (1985) and Santhanakrishnan (1987) who reported prevalence rates of Shigella to be 20.6% and 22% respectively in their studies. The difference in isolation rate varies according to epidemiological setting of study.

The isolation rate of Shigella in the present study was lowest below 6 months of age. The isolation rates were higher after infancy, with peak during 2 to 3 years of age. Santhanakrishnan (1987) have reported that children under 2 years are predominant victims, 80% cases in his study were children under 2 years of age. Various other studies have reported Shigellesis to be common during weaning period. But number of shigella in present study

is very small to elaborate any valid association regarding age distribution.

Stoll et al (1982) in their study found lowest incidence of Shigella during infancy and attributed it to probable protective effect of breast feeding or children in infancy has less exposure.

Salmonella as a causative agent of acute diarrhoea has remained relatively of less importance (Sanyal, 1981). Nontyphoid Salmonella like S. typhimurium, S. newport etc. have been implicated in both endemic and epidemic situations (Paul et al, 1981; Fule et al, 1985). In the present study isolation of Salmonella was low .16% which is consistent with many Indian studies (Bhan et al, 1987 - 2.5%, Sen et al, 1985 - 0.9%; Mohandas et al, 1987 - 3%). Sanyal et al (1977) in their study could not isolate Salmonella from any case of acute diarrhoea. S. typhimurium was the commonest serotype in various studies (Saxena et al, 1981; Fule et al, 1985 and Bhat et al, 1985).

Out of 3 Salmonella isolates 2 were present below 6 months of age and 1 in 13-24 months age group. The number of Salmonella is too small to make any valid association with the age distribution.

With the development of practical methods of isolation of C. jejuni, from faeces, this organisms has been gaining increasing recognition as an important enteric pathogen (Nayyar et al, 1983). In the present study Campylobacter was isolated from 5.8% cases of acute

diarrhoea. Many studies from developing and developed world have reported the isolation rates between 4 to 15 percent(Pai et al, 1979; Blaser et al, 1980; Nayyar et al, 1983; Bhan et al, 1987; Nair et al, 1985 and Mohandas et al, 1987). In the present study isolation rate of Campylobacter in acute diarrhoeal children is lower than many Indian studies (Nayyar et al, 1983 - 10.3%; Bhan et al, 1987 - 10.2%; Mohandas et al, 1987 - 13%). Whereas many other authors have reported relatively lower isolation rates (Khatua et al, 1984-2%; Bhat et al, 1985-3.2%; and Sen et al, 1985 - 2.6%).

In the present study difference between isolation rates of C. jejuni from patients with diarrhoea and from controls is not significant. This is in agreement with most of the studies from India and Bangladesh. Studies by Blaser et al (1980) from Bangladesh reported almost equal isolation rates from diarrhoeal and healthy children (12% and 14%). Rajan et al (1982) have shown higher excretion rate of C. jejuni from healthy children and adults in southern India. This high excretion rate of C. jejuni in nondiarrhoeal individuals makes it difficult to enterpret exact significance of C. jejuni in causation of diarrhoea.

In the present study isolation rate of C. jejuni was maximum during 7-24 months of age group and isolation rates were lower before 6 months and after 2 years. In various studies where C. jejuni has been found to be more

common in lower age group (Stoll et al, 1982, Nair et al, 1984 and Blaser et al, 1980).

E. coli forms a part of normal flora of the gut but producing no disease. Normally several intricate mechanisms confine E. coli to the large intestine and prevent spread to the proximal bowel. As a result of breakdown of natural defences, a complex series of interactions between external environment, the host and organisms leads to extensive colonization of small gut by E. coli predisposing individual to syndrome of diarrhoea (Harris, 1976). Large scale proliferation of these organisms is demonstrated as pure or predominant growth on primary culture of stool.

In the present study E. coli was cultured as a pure or predominant growth in 21.8%. Children with acute diarrhoea and in 11% non-diarrhoeal controls. Agarwal et al (1980) reported E. coli as a predominant growth in 60.6% cases of acute diarrhoea in young children out of which only 21.2% were typable. Sarkar et al (1980) documented E. coli in pure or predominant growth in 37% cases of acute diarrhoea out of which 58.8% were typable strains. Paul et al (1980) isolated E. coli in a pure culture in 30.6% cases and 22.6% controls. In the present study isolation of E. coli as predominant growth culture was lower in comparison to previous studies. This may be because many patients might have taken antibiotics before first contact in our out patient clinic.

To day it has became an acceptable practice to distinguish E. coli that cause diarrhoea into three types - Enterotoxigenic E. coli, enteropathogenic E. coli and enteroinvasive E. coli (Merson and Black , 1981). In the last two decade most of the studies on etiology of acute diarrhoea have included test for enterotoxigenicity for E. coli. In the present study due to certain constrains like non-availability of the typing antisera, and lack of laboratory facilities for testing enterotoxigenicity, we could not screen for E. coli strains as EPEC and ETEC. Hence our results regarding incidence in the present study is not comparable with the studies conducted in last two decades.

Isolation rate of Entamoebahistolytica and Giardia lamblia remained low in present study. G. lamblia and E. histolytica were isolated in 3.2% and 2.7% cases respectively. Only in the presence of trophozoites in stools examination, these organisms were considered possible diarrhoeal pathogen. Lower isolation of G. lamblia and E. histolytica in present study are consistent with studies of Sen et al (1981).& Bhan et al (1987). Mohandas et al (1987) reported 5% prevalence of G. lamblia in acute diarrhoea in young children. In present study G. lamblia and E. histolytica were not isolated in infancy, isolation rate was relatively higher after two years of age. However, sample size is very small to consider any association with age distribution.

Studies from Bangladesh have documented that diarrhoea associated with these two organisms is more common in older children suggesting short lived transplacental immunity, protection due to breast feeding or decreased exposure in infancy (Stoll et al, 1982).

The importance of breast feeding for the health of young children has been recognised for long time. The breast feeding has a major beneficial effect upon infection, malnutrition and unregulated fertility. The beneficial effect of breast feeding has been demonstrated by many extensive studies. Cunningham et al (1977) have shown that breast fed babies are less prone to development of enteral and other infections. Fallot et al (1980) have demonstrated that the incidence of breast feeding in hospitalised infants was 11% compared to expected figures of 25.2% in community in the same group. This shows that breast fed infants were less prone to development of serious illnesses.

Kumar et al (1981) in a longitudinal study have clearly demonstrated lower morbidity among breast fed infants in a community. Bhatia et al (1981) observed in hospital based study that bottle fed babies dominated the diarrhoeal group as compared to non diarrhoeal group. Mittal et al (1983) in their hospital based study also demonstrated that prevalence of bottle feeding was significantly higher in diarrhoeal group when compared with non-diarrhoeal group.

In the present study we observed that prevalence

of breastk feeding was apparently higher in non-diarrhoeal group as compared to diarrhoeal group in children under two years. But this difference failed to reach the level of statistical significance ($p > 0.05$). However, in infants below 6 months the number of exclusively breast fed babies was significantly ($p < 0.01$) higher in non-diarrhoea group than that of diarrhoeal group. Thus it may be interpreted that breast feeding provides protection against diarrhoeal diseases. The protective role of breast feeding is discernible in younger infants in the present study.

In the present study an attempt was made to see the correlation between micro-organisms isolated in stool examination of exclusively breast fed and bottle fed (partly or solely) children of diarrhoeal group. It was observed that all the major micro-organisms i.e. rotavirus E. coli, Shigella, Salmonella and Campylobacter were isolated in both the groups. Isolation rates of Shigella and Campylobacter are apparently higher in bottle fed infants, but this difference was not statistically significant. E. coli and rotavirus were isolated in almost similar proportion from bottle fed and breast fed patients.

It has been well shown in various studies that incidence of diarrhoeal disease is lower in breast fed infants. This decrease is attributed mainly to prevention of bacterial diarrhoeas. Fallot et al (1980) failed to find any bacterial pathogens among hospitalised diarrhoeal infants who had been breast fed in a nontropical setting.

However, Cushing et al (1982) failed to find any protection against toxigenic E. coli among breast fed infants. It has been suggested that protection against E. coli infection may depend upon presence of specific E. coli antibodies in breast milk of the mother. Mittal et al (1983) in their hospital based study observed that there was no difference in enteropathogenic organisms isolated from differently fed children. E. coli, Salmonella and Shigella were isolated frequently in exclusively breast fed infants. The authors attributed their findings to heavy environmental contamination prevaling in our setting.

Weinberg et al (1984) have shown that incidence of rotavirus infection, its average age of occurrence and severity among differently fed infants are largely comparable. Samantray et al (1982) also observed that breast feeding provides only partial protection. In the present study no difference was observed in incidence of rotavirus detection in bottle fed (partly or solely) and breast fed patients. Thus our study is in agreement with study of Weinberg et al (1984).

It is noteworthy that in exclusively breast fed infants bacterial enteropathogens like E. coli, and Shigella and Salmonella were frequently isolated. This could be because in hot climatic conditions even young infants are often offered water, which is not boiled. And usually water in our set up is contaminated. Many parents use bottle for this purpose, which is another source of

heavy contamination. Thus the protective effects of breast feeding are probably overwhelmed by environmental condition in very poor socio-economic areas.

It has been suggested that determining factor in high prevalence of diarrhoeal disease in developing areas of the world would appear to be heavily contaminated environment, particularly water and weaning foods given to infants, and breast feeding in these areas can provide a significant but not absolute protection against diarrhoeal disease (Mittal, 1986).

Diarrhoea and malnutrition are commonly associated. Diarrhoea may affect nutritional status in several ways, decreased food intake (Molla et al (1986), withholding food as a measure to control diarrhoea, loss of micro and macronutrients, and catabolic response. On the other hand malnutrition also leads to increased prevalence of diarrhoeal disease. Ghai and Jaiswal (1970) have shown increased attack rate of diarrhoeal disease in malnourished children. However, a study from Bangladesh has failed to show increase in attack rate (Chen et al, 1981). In a prospective study Chen et al (1981) suggested that the impact of diarrhoea in predisposing and exacerbating malnutrition may be most important of the bidirectional effect. The prominent effect of the malnutrition on diarrhoea may be through disease duration and mortality rather than through disease incidence.

In our study there is preponderance of malnourished children. 67.2% children in the present study are under

80% of the expected reference weight for age. A nutritional anthropometric study conducted in rural areas of Jhansi, from where most of our patients belonged, revealed that 82% preschool children were below Harvard standard of weight for height (Verma et al, 1980).

In the present study we analysed micro-organisms isolated in stool examination according to nutritional status. The isolation rates of various aetiolotic agents in normal, mild and severely malnourished children were 56.4%, 65.9% and 68.6% respectively. This difference in proportion of isolated organisms between normal and severely malnourished is not significant. Rotavirus and E. coli were isolated in almost equal frequency in normal and severely malnourished children. However, Shigella was apparently higher in severely malnourished children (11.4% Vs 8.6%). But the difference is not statistically significant.

Samantray et al (1984) in their study reported no difference in rotavirus detection in normal nourished and malnourished children. Stoll in his extensive study documented that there was no significant difference nutritional status between children with shigellosis and those without. Mittal et al (1981) reported that isolation rate of bacterial enteropathogen from severely malnourished children were significantly higher as compared to normal nourished children. More definitive pathogens like Shigella Salmonella and Klebsiella were isolated more frequently from severely malnourished children.

The clinical features of acute diarrhoeal disease have been well documented. There is considerable overlap in the clinical features of diarrhoea attributed to different micro-organisms, and simultaneously there is variation in clinical features attributed to same organism. Clinically acute diarrhoea tend to present either as acute watery diarrhoea, with semiformed or semiliquid stools, or as dysentry i.e. blood in stools with or without mucous. It may be accompanied by vomiting, pain in abdomen, tenensmus, fever, concurrent respiratory symptoms etc.

In the present study watery diarrhoea was presenting symptom in 48.7% of the total patients studied. Invasive diarrhoea defined as presence of blood in stools with or without mucous was the chief complaint in 13.8%. of total patients with acute diarrhoea. Stoll et al(1982) in their extensive study in Bangladesh reported 65% of acute diarrhoeas were watery and 20% were invasive in nature. Commonest organism in causation of watery diarrhoea are rotavirus, ETEC and V. cholerae (Stoll et al, 1982; Black et al, 1981). Jain et al (1983) and Mohandas et al (1989) in their study reported incidence of acute watery diarrhoeas to be 50.6% and 47% and of invasive diarrhoea 17.5% and 7% respectively.

Vomiting is an important complaint which may be associated with acute diarrhoea. 39.2% patients in present study had vomiting. Stoll et al (1982) reported vomiting in 58% of total patients attending hospital. Larger

proportion vomiting in their study could be due to high prevalence of ETEC and V. cholerae. Mohandas et al (1987) reported vomiting in 37.6%, there is high degree of association in vomiting and rotavirus illness. ETEC and V. cholerae diarrhoea are also reported to be associated with more frequent vomiting (Stoll et al, 1982).

In the present study fever was observed in 40.7% cases. The presence of fever was more frequently associated with Shigella ($p < 0.05$). Various studies have reported concurrent upper respiratory tract infection with acute diarrhoea. The percentage of U.R.I. is quite variable in different studies. Some western studies have reported higher incidence of URI with rotavirus diarrhoea (Lewis et al, 1979). However many Indian studies have failed to find such association (Samantray et al, 1982; Mohandas et al, 1987). In present study evidence of upper respiratory tract infection was observed in 39.1% of total patients.

Dehydration is universally known most important consequence. In present study 46% (87 out of 189) patients were dehydrated. 22.2% had mild, 14.8% moderate and 9% had severe dehydration. 56(29.6%) patients were admitted in pediatric ward for management

Clinical illness attributed to rotavirus is typically described as watery diarrhoea generally with prominent vomiting and not uncommonly with fever and respiratory symptoms. In the present study watery stools

and vomiting were present in 82.3% and 70.6% cases respectively. A high degree of association between rotavirus aetiology and watery diarrhoea ($p < 0.01$) and with vomiting ($p < 0.01$) was found . The more frequent occurrence of watery diarrhoea and vomitings with rotavirus have been well documented in literature (Shephered et al, 1975; Rodriquez et al, 1977; Black et al, 1981; Stoll et al, 1982 and Mohandas et al, 1987).

Blood in stools is rare in rotavirus diarrhoea mucous in stool may be present in upto 25% cases (WHO Scientific group). In present study blood was not observed in any stool sample however 17.6% children were having mucous in their stool.

Fever in association with rotavirus have been found to be more common in some studies (Stoll et al,1982). However, earlier reports from west (Rodriquez et al, 1977) did not show any such significant association. We in present study could not find significant association ($p > 0.05$) of rotaviral illness with URI. Lewis et al (1979) documented significant association of URI with rotavirus diarrhoea. However, some Indian studies (Samantray et al, 1982; Mohandas et al, 1987) did not find any significant association. In present study also does not show significant association ($p > 0.05$) between rotavirus illness and upper respiratory tract infection.

In the present study patients with Shigella infection presented with both, watery diarrhoea and

dysentry. Presence of blood in stool (66.7%), mucous (73.4%) and pain in abdomen (46.6%) was present more frequently in Shigella infection. A statistically significant association of these features with shigellosis was observed. Fever was also present more frequently with Shigella infection. Presence of vomiting was relatively infrequent. Thus the presence of blood and mucous in stools and pain in abdomen indicate towards shigella aetiology. Though the sample size of study is small the results are in agreement with study carried out in Bangladesh (Stoll et al,1982). Shigella produces diarrhoea by two mechanisms by toxin production affecting small bowel resulting in watery diarrhoea and by tissue invasion of colon resulting in classical dysentry. The patients with watery diarrhoea have more vomiting and more severe dehydration as compared to patients who present with dysentry (Stoll et al, 1982; Dutta et al, 1989).

Prolapse rectum have been reported to be common in shigellosis. Santhanakrishanan et al (1987) reported prolapse rectum in nearly half of the patients with shigellosis. In the present study we observed prolapse rectum in 20.0% patients. Serious complications like secondary sepsis which increase the morbidity and mortality in shigellosis (Alam et al, 1984) were not observed in our study.

Campylobacter was isolated in 11 patients with acute diarrhoea in present study. 6 patients (54.5%) had watery diarrhoea, 3(27.3%) patients had invasive diarrhoea. Mucoid stools were present in 36.4% and pain in abdomen was present in 4 patients (36.4%). In half of our patients watery diarrhoea was present. Nayyar et al(1983) have also reported watery diarrhoea in nearly half of their patients and [illegible] invasive features in one fourth patients. Our study is in agreement with the study conducted by Nayyar et al (1983). A high association with watery diarrhoea and campylobacteriosis was reported by Bhattacharya et al (1985) from Calcutta. Studies from Bangladesh (Blaser et al, 1980 and Stoll et al, 1982) have documented more frequent association of watery diarrhoea with campylobacteriosis. Reports from developed countries, however, have reported dysentric syndrome more frequently associated with campylbacter diarrhoea (Butzler, 1981). Stoll et al(1982) in their study in Bangladesh documented that watery diarrhoea mucoid stools, pain in abdomen were more frequently present. However, in the present study the number of C. jejuni is too small to elaborate an association with many clinical features.

SUMMARY AND CONCLUSION

SUMMARY AND CONCLUSION

The present work entitled "A clinico-microbiological study of acute diarrhoea in children" was carried out in the department of Pediatrics, M.L.B. Medical College, Jhansi. A total of 189 children under the age of 5 years with acute diarrhoea were studied. One hundred age and sex matched non-diarrhoeal children comprised the control group. Children with acute diarrhoea were selected from Paediatric out patient Clinic. Detailed history, in particular, for duration of illness, frequency and consistency of stools, presence of blood and mucous in the stools, associated feature like vomiting, fever, pain in abdomen, tenensmus etc. was recorded. Feeding pattern was noted in children under 2 years of age with emphasis on breast feeding, bottle feeding and bottle hygiene. A detailed physical examination was carried out. Assessment of nutritional status was done according to classification of Indian Academy of Pediatrics.

Stool samples were collected in clean glass vials and were sent to the laboratory within two hours of collection. In the laboratory samples were processed immediately.

A naked eye examination of stool samples for consistency and presence of blood and mucous was done. A direct microscopic examination was carried out for detection of trophozoites of G. lamblia and E. histolytica

Stool specimens were processed for the isolation of bacterial enteropathogens including campylobacter jejuni. For isolation of campylobacter, Blaser's medium was used. Due to non-availability of typing antisera and laboratory constrains, screening E. coli strains (as pure or predominant ggrowth) could not be performed for enteropathogenic E. coli (EPEC) and enterotoxigenic E. coli (ETEC). Detection of rotavirus antigen in faecal specimen was done by enzyme linked - immunosorbant assay (ELISA) using methodology and reagents supplied by WHO collaborating centre on rotaviruses. Birmingham, U.K. Reading of ELISA was taken by naked eye.

On stool examination, enteropathogens were found in 65.1% children with acute diarrhoea. Rotavirus was detected using ELISA technique in 18% children with acute diarrhoea. Bacterial enteropathogens belonging to 6 species were isolated in 40.7% of diarrhoeal cases. Bacterial enteropathogens found were E. coli in 21.7%, Shigella in 7.9%, Klebsiella in 1.1%, Campylobacter in 5.8%, Salmonella in 1.6%, and Pseudomonas in 1.1%. G. Lamblia and E. histolytica were found in 3.2% and 2.6% cases respectively. Mixed agents were detected in 2.1% cases.

Enteropathogenic organisms were encountered in 17% of non diarrhoeal controls. E. coli were isolated in 11%, Campylobacter in 4%, mixed pathogens in 1% and rotavirus in 1% non diarrhoeal control children. Shigella, Klebsiella and Pseudomonas and trophozoites of G. lamblia, E. histolytica were not encountered in control children.

Vibrio-cholerae and vibrio-parahaemolyticus were not isolated from any case or control in the present study. Isolation rate of campylobacter was not significantly different in diarrhoeal and non diarrhoeal children.

Rotavirus diarrhoea occurred mostly in children below 2 years, peak during 7-12 months. No rotavirus was detected after 3 years of age. Shigella infection was less frequent during infancy. Campylobacter was found more frequently during 7-24 months of age.

An analysis of feeding pattern of diarrhoeal and non diarrhoeal children under 2 years revealed that substantial number of children in both the groups were bottle fed. The prevalence of breast feeding below 6 months of age was significantly higher in nondiarrhoeal group as compared to diarrhoeal(84.2% Vs 55.9%; $p < 0.01$). The enteropathogens isolated from stool examination in breast fed and bottle fed were largely comparable.

There was preponderance of malnourished children in the present study. 67.2% children in diarrhoeal group were undernourished..19% of total diarrhoeal children were suffering from grade III and IV malnutrition. No significant difference was observed in isolation of different enteropathogens in normally nourished and severely malnourished children.

Out of total 189 children with acute diarrhoea nearly half (48.7%) presented with watery diarrhoea. 13.8% children had blood in stool with or without mucous.

Children with rotavirus illness presented more frequently with watery diarrhoea (82.3%) and vomiting (70.6%). Presence of fever and upper respiratory tract infection was NOT found to be more frequently associated. In E. coli diarrhoea clinical features were not specific. Half of children campylobacteriosis presented with watery diarrhoea. Children with Shigella infection presented both as dysentry and watery diarrhoea. But dysentry was more frequent (66.7%) with Shigella infection.

The following conclusions were drawn from the present study.

1. Rotavirus is an important cause of acute diarrhoea in children particularly below 2 years of age. Rotavirus accounted for 18% diarrhoeas in children under 5 years of age in the present study.
2. Bacterial enteropathogens accounted for 40.7% cases of diarrhoea. Commonest among them were E.coli (21.7%), Shigella (7.9%) and Campylobacter (5.8%).
3. There was no significant difference in isolation rates of campylobacter from diarrhoeal and non diarrhoeal children.
4. Diarrhoea due to vibrio-cholerae is probably rare in this region as V. cholerae was not detected in any case in the present study.
5. G. lamblia and E. histolytica were not common as a cause of acute diarrhoea in children.

6. Protective role of breast feeding is discernible in young infants.

7. There was no significant difference notable in microorganisms isolated in stool examination of breast fed and bottle fed cases and similarly in normally nourished and undernourished children with acute diarrhoea.

8. Clinical features of rotavirus and shigella diarrhoea may help in indicating towards their etiology.

B I B L I O G R A P H Y

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